(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 016 407 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
10.05.2006 Bulletin 2006/19

(21) Application number: **98941725.8**

(22) Date of filing: **04.09.1998**

(51) Int Cl.:
A61K 31/52 *(2006.01)* ***C07D 473/04*** *(2006.01)*
A61P 9/10 *(2006.01)*

(86) International application number:
PCT/JP1998/003980

(87) International publication number:
WO 1999/012546 (18.03.1999 Gazette 1999/11)

(54) **XANTHINE DERIVATIVES FOR TREATING BRAIN ISCHEMIA**

XANTHINDERIVATIVE ZUR BEHANDLUNG VON HIRNISCHÄMIE

DERIVES DE LA XANTHINE CONTRE L'ISCHEMIE CEREBRALE

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **05.09.1997 JP 24056597**

(43) Date of publication of application:
05.07.2000 Bulletin 2000/27

(60) Divisional application:
06005220.6

(73) Proprietor: **KYOWA HAKKO KOGYO CO., LTD.**
Chiyoda-ku,
Tokyo 100-8185 (JP)

(72) Inventors:
- **SHIMADA, Junichi,**
  Royal Hills Nibankan No 305
  Sunto-gun,
  Shizuoka 411-0933 (JP)
- **KUROKAWA, Masako**
  Mishima-shi,
  Shizuoka 411-0815 (JP)
- **IKEDA, Ken**
  Sunto-gun,
  Shizuoka 411-0944 (JP)
- **SUSUKI, Fumio**
  Mishima-shi,
  Shizuoka 411-0021 (JP)
- **KUWANA, Yoshihisa**
  Sunto-gun,
  Shizuoka 411-0943 (JP)

(74) Representative: **Vossius & Partner**
Siebertstrasse 4
81675 München (DE)

(56) References cited:
EP-A- 0 374 808
EP-A- 0 389 282
EP-A- 0 565 377
EP-A- 0 590 919
EP-A- 0 607 607
EP-A- 0 698 607
DE-A- 4 236 331
JP-A- 6 239 862
JP-B2- 2 613 352
JP-B2- 2 613 355
JP-B2- 6 047 539
US-A- 4 772 607
US-A- 5 173 491

- **HEESE K ET AL: "Nerve growth factor (NGF) expression in rat microglia is induced by adenosine A2a-receptors." NEUROSCIENCE LETTERS. IRELAND 8 AUG 1997, vol. 231, no. 2, 8 August 1997 (1997-08-08), pages 83-86, XP002205285 ISSN: 0304-3940**
- **CANHAO P ET AL: "1,3-DIPROPYL-8-CYCLOPENTYLXANTHINE ATTENUATES THE NMDA RESPONSE TO HYPOXIA IN THE RAT HIPPOCAMPUS" BRAIN RESEARCH, AMSTERDAM, NL, vol. 1/2, no. 661, 1994, pages 265-273, XP008004873 ISSN: 0006-8993**
- **LUBITZ VON D K J E ET AL: "CHRONIC ADMINISTRATION OF SELECTIVE ADENOSINE A1 RECEPTOR AGONIST OR ANTAGONIST IN CEREBRAL ISCHEMIA" EUROPEAN JOURNAL OF PHARMACOLOGY, AMSTERDAM, NL, vol. 2, no. 256, 21 April 1994 (1994-04-21), pages 161-167, XP008004867 ISSN: 0014-2999**

EP 1 016 407 B1

(Cont. next page)

- PHILLIS J W: "THE EFFECTS OF SELECTIVE A1 AND A2A ADENOSINE RECEPTOR ANTAGONIST ON CEREBRAL ISHCEMIC INJURY IN THE GERBIL" BRAIN RESEARCH, AMSTERDAM, NL, vol. 705, no. 1/2, 24 December 1995 (1995-12-24), pages 79-84, XP001006295 ISSN: 0006-8993
- MACGREGOR D G ET AL: "BLOCKADE BY 1,3-DIPROPYL-8-CYCLOPENTYLXANTHINE (CPX) OF PURINE PROTECTION AGAINST KAINATE NEUROTOXICITY" BRAIN RESEARCH, AMSTERDAM, NL, vol. 644, no. 2, 1994, pages 339-342, XP001006257 ISSN: 0006-8993
- DALPIAZ A ET AL: "DE NOVO ANALYSIS OF RECEPTOR BINDING AFFINITY DATA OF 8-ETHENYL-XANTHINE ANTAGONISTS TO ADENOSINE A1 AND A2A RECEPTORS" ARZNEIMITTEL FORSCHUNG. DRUG RESEARCH, EDITIO CANTOR. AULENDORF, DE, vol. 5, no. 47, 1997, pages 591-594, XP001073979 ISSN: 0004-4172
- DATABASE MEDLINE [Online] 1996 MALLY J ET AL: "Potential role of adenosine antagonist therapy in pathological tremor disorders." Database accession no. NLM9364577 XP002205263 & PHARMACOLOGY & THERAPEUTICS. ENGLAND 1996, vol. 72, no. 3, 1996, pages 243-250, ISSN: 0163-7258
- KANDA T ET AL: "ADENOSINE A2A RECEPTORS MODIFY MOTOR FUNCTION IN MPTP-TREATED COMMON MARMOSETS" NEUROREPORT, RAPID COMMUNICATIONS OF OXFORD, OXFORD, GB, vol. 9, no. 12, 24 August 1998 (1998-08-24), pages 2857-2860, XP001006279 ISSN: 0959-4965
- KANDA T ET AL: "ADENOSINE A2A ANTAGONIST: A NOVEL ANTIPARKINSONIAN AGENT THAT DOES NOT PROVOKE DYSKINESIA IN PARKINSONIAN MONKEYS" ANNALS OF NEUROLOGY, BOSTON, US, vol. 4, no. 43, 1998, pages 507-513, XP008004872 ISSN: 0364-5134
- ISHIMARU H.: 'NGF delays rather than prevents the cholinergic terminal damage and delayed neuronal death in the hippocampus after ischemia' BRAIN RES. vol. 789, no. 2, 1998, pages 194 - 200
- KUMAR K. ET AL EFFECT OF HYPOTHERMIA ON MICROGLIAL REACTION IN ISCHEMIC BRAIN vol. 8, no. 4, March 1997, pages 947 - 950
- KARPIAK S.: 'Gangliosides (GM1 and AGF2) reduce mortality due to ischemia: protection of membrane function' STROKE vol. 18, no. 1, 1987, pages 184 - 187

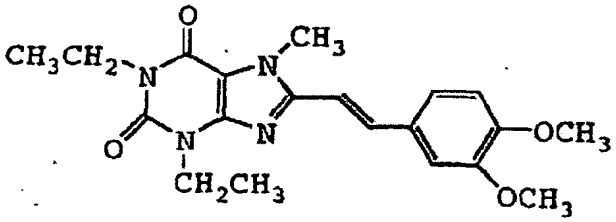
Description

[0001] The present invention relates to a therapeutic agent for neurodegenerative disorders.

[0002] Most of the compounds used according to the present invention are known compounds, and their adenosine A_2-receptor antagonism, anti-Parkinson's disease action, anti-depressive action, anti-asthmatic action, inhibitory action on bone absorption and action on central excitation are known [Japanese Published Examined Patent Application No. 26516/72, J. Med. Chem., 34, 1431 (1991), J. Med. Chem., 36, 1333 (1993), WO 92/06976, Japanese Published Unexamined Patent Application No. 211856/94, Japanese Published Unexamined Patent Application No. 239862/94, WO 95/23165, Japanese Published Unexamined Patent Application No. 16559/94 and WO 94/01114).

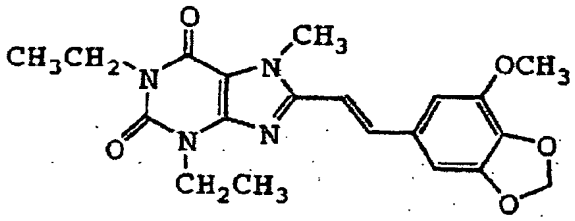
[0003] However, it is not known that said compounds have an inhibitory action on neurodegeneration.

[0004] The present invention relates to the use of a xanthine derivative selected from a compound of formula (1):



(1)

and a compound of formula (2)



(2)

or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of brain ischemia.

[0005] In a preferred embodiment, the xanthine derivative is represented by formula (1), or a pharmaceutically acceptable salt thereof.]

[0006] The pharmaceutically acceptable salts of compounds of formulae (1) and (2) include pharmaceutically acceptable acid addition salts, metal salts, ammonium salts, organic amine addition salts and amino acid addition salts.

[0007] The pharmaceutically acceptable acid addition salts of compound of formulae (1) and (2) include inorganic acid addition salts such as hydrochloride, sulfate and phosphate, and organic acid addition salts such as acetate, maleate, fumarate, tartrate, citrate and methanesulfonate; the pharmaceutically acceptable metal salts include alkali metal salts such as sodium salt and potassium salt, alkaline earth metal salts such as magnesium salt and calcium salt, aluminum salt, and zinc salt; the pharmaceutically acceptable ammonium salts include ammonium and tetramethylammonium; the pharmaceutically acceptable organic amine addition salts include salts with morpholine and piperidine; and the pharmaceutically acceptable amino acid addition salts include salts with lysine, glycine and phenylalanine.

[0008] Compounds of formulae (1) and (2) can be produced by the methods disclosed in the above-mentioned publications or according to the methods. The desired compound in the process can be isolated and purified by purification methods conventionally used in synthetic organic chemistry, such as filtration, extraction, washing, drying, concentration, recrystallization and various kinds of chromatography.

[0009] In the case where a salt of compound formulae (1) and (2) is desired and it is produced in the form of a desired salt, it may be subjected to purification as such. In the case where compounds of formulae (1) and (2) are produced in the free form and their salt is desired, they are dissolved or suspended in a suitable solvent, and then an acid or a base may be added thereto to form the salt.

[0010] Compounds of formulae (1) and (2) and pharmaceutically acceptable salts thereof may be in the form of adducts with water or various solvents, which can satisfactorily be used as the therapeutic agent of the present invention.

[0011] Some of compounds of formulae (1) and (3).

[0012] Further details of compounds of formulae (1) and (2) are shown below.

Compound 1: (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (Japanese Published Unexamined Patent Application No. 211856/94)
Melting point: 190.4-191.3 °C
Elemental analysis: $C_{20}H_{24}N_4O_4$
Calcd. (%): C 62.48, H 6.29, N 14.57
Found (%): C 62.52, H 6.53, N 14.56
IR(KBr) vmax(cm^{-1}): 1697, 1655, 1518
NMR(CDCl3, 270MHz) δ(ppm): 7.74(1H, d, J=15.5Hz), 7.18(1H, dd, J=8.3, 1.9Hz), 7.08(1H, d, J=1.9Hz), 6.89(1H, d, J=8.3Hz), 6.77(1H, d, J=15.5Hz), 4.21(2H, q, J=6.9Hz), 4.09(2H, q, J=6.9Hz), 4.06(3H, s), 3.96(3H, s), 3.93(3H, s), 1.39(3H, t, J=6.9Hz), 1.27(3H, t, J=6.9Hz)

Compound 2: (E)-1,3-diethyl-8-(3-methoxy-4,5-methylenedioxy styryl)-7-methylxanthine (Japanese Published Unexamined Patent Application No. 211856/94)
Melting point: 201.5-202.3 °C
Elemental analysis: $C_{20}H_{22}N_4O_5$
Calcd. (%): C 60.29, H 5.57, N 14.06
Found (%): C 60.18, H 5.72, N 13.98
IR(KBr) vmax(cm^{-1}): 1694, 1650, 1543, 1512, 1433
NMR(DMSO-d_6, 270MHz) δ (ppm) : 7.58 (1H, d, J=15.8Hz), 7.23(1H, d, J=15.8Hz), 7.20(1H, d, J=1.0Hz), 7.09(1H, d, J=1.0Hz), 6.05(2H, s), 4.09-4.02(2H, m), 4.02(3H, s), 3.94-3.89 (2H, m), 3.89(3H, s), 1.25(3H, t, J=7.2Hz), 1.13 (3H, t, J=6.9Hz)

[0013] Hereinafter, the pharmacological activity of compound 1 is shown by the following Test Examples.

Test Example 1: Inhibitory Action on Neurodegeneration

[0014] The experiment was conducted according to the method of Sundström et al. (Brain. Res. Bulletin, 21, 257-263 (1988))

[0015] In the experiment, 9- to 10-week-old male C57BL/6NCrj mice (supplied by Nippon Charles River) were used. During the period of preliminary breeding, the animals were kept in a laboratory at room temperature (22 to 24 °C) under 50 to 60 % humidity and allowed food and water ad libitum

[0016] 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (abbreviated hereinafter as MPTP HCl (RBI Co., Ltd.)) was dissolved at a concentration of 4 mg/ml in physiological saline. A test compound was suspended at a concentration of 1 mg/ml in 0.3 % dimethyl sulfoxide (DMSO). Each test group consisted of 9 to 10 animals, and a control group was intraperitoneally given physiological saline, and an MPTP HCl administration group and an MPTP HCl + test compound administration group were intraperitoneally given MPTP HCl (40 mg/kg)

[0017] After 1 hour, the control group and the MPTP HCl administration group were orally given 0.3 % Tween, and the MPTP HCl + test compound administration group was orally given the test compound (10 mg/kg). After 1 week, the animals were decapitated, and the striatum was removed therefrom under cooling on ice. The striatum was stored in a deep freezer (< -80°C) before a binding experiment.

[0018] A [^{3}H]-mazindol binding test was conducted in the following method. A striatum and 300 μl of buffer (120 mM NaCl, 5 mM KCl, 50 mM Tris, pH 7.9) were put into a micro-centrifuge tube and homogenized by portable homogenizer S-203 (manufactured by Iuchi and centrifuged at 15,000 rpm, 4°C for 5 minutes (by KUBOTA 1710). The precipitates were suspended in 300 μl of buffer and then centrifuged again at 15,000 rpm, 4°C for 5 minutes. The precipitates were suspended in 500 μl of buffer and then distributed into four test tubes in 100 μl portions. The remaining suspension (100 μl) was used for protein quantification. To determine non-specific binding, nomifensine maleate (RBI Co., Ltd.) (final concentration: 10 μM) as an inhibitor of dopamine uptake was added to two test tubes among the four test tubes. The binding reaction was initiated by adding 25 μl of [^{3}H]-mazindol (final concentration: 10 nM) (Spec. Act. 888 GBq/mmol, a product of NET). The mixture was incubated for 1 hour under cooling on ice, and the striatum homogenate was adsorbed onto a glass filter (Whatman, GF/B) in a cell harvester and washed three times with 5 ml of buffer. The radioactivity on the glass filter was measured with a liquid scintillation counter. For each striatum, specific [^{3}H]-mazindol binding was determined by subtracting the average of non-specific [^{3}H]-mazindol binding from the average of total [^{3}H]-mazindol binding.

[0019] Protein quantification was conducted by use of a Bio-Rad DC protein assay kit (Bio-Rad Co., Ltd.) with bovine serum albumin (Sigma Co., Ltd.) as a standard. Specific [^{3}H]-mazindol binding was expressed as the amount of bound [^{3}H]-mazindol per unit weight of protein, and the mean ± standard error was determined for each group (9 to 10 animals).

[0020] In Table 1, the results are expressed in terms of the amount of specifically bound [^{3}H]-mazindol (fmol/mg protein) in the striatum.

Table 1

Test groups	
Control	1140.3±50.0
MPTP HCl	616.3±32.8###
MPTP HCl + compound 1	950.9±54.1***
Control	1219.3 ± 66.4
MPTP HCl	621.2±27.7###
MPTP HCl + compound 2.	794.9±28.5**

**: $p < 0.01$ (compared with the group given MPTP HCl alone).
***: $p < 0.001$ (compared with the group given MPTP HCl alone).
###: $p < 0.001$ (compared with the control group).
(n = 9 to 10; Wilcoxon rank sum test)

[0021] According to the test results, the reduction of the amount of specifically bound [^{3}H]-mazindol by administration of MPTP HCl was inhibited by compound 1. That is, it was revealed that compound 1 exhibits inhibitory action on degeneration of dopaminergic neurons.

Test Example 2: Acute Toxicity Test

[0022] Test compounds were orally or intraperitoneally administered to groups of ddy-strain male mice weighing 20 ± 1 g, each group consisting of three mice. Seven days after the administration, the mortality was observed to determine a minimum lethal dose (MLD) of each compound.

[0023] The MLD value of Compound 1 was greater than 1000 mg/kg for oral administration.

[0024] Compounds of formulae (1) and (2) or pharmaceutically acceptable salts thereof have inhibitory action on neurodegeneration and are useful as a therapeutic agent for the neurodegenerative disorder.

[0025] Compounds of formulae (1) and (2) or pharmaceutically acceptable salts thereof can be used as such or in the form of various pharmaceutical compositions. The pharmaceutical compositions used according to the present invention can be prepared by uniformly mixing an effective amount of compounds of formulae (1) or (2) or a pharmaceutically acceptable salt thereof as an active ingredient with pharmaceutically acceptable carriers. The pharmaceutical compositions are preferably in a unit dosage form suitable for rectal administration, oral or parenteral (including subcutaneous, intravenous and intramuscular administration) administration, etc.

[0026] For preparing a pharmaceutical composition for oral administration, any useful pharmaceutically acceptable carriers can be used. For example, liquid preparations for oral administration such as suspension and syrup can be prepared using water; sugars such as sucrose, sorbitol and fructose; glycols such as polyethylene glycol and propylene glycol; oils such as sesame oil, olive oil and soybean oil; preservatives such as a p-hydroxybenzoate; flavors such as strawberry flavor and peppermint, etc. Powder, pills, capsules and tablets can be prepared using excipients such as lactose, glucose, sucrose and mannitol; disintegrating agents such as starch and sodium alginate; lubricants such as magnesium stearate and talc; binders such as polyvinyl alcohol, hydroxypropyl cellulose and gelatin; surfactants such as fatty acid esters; plasticizers such as glycerin, etc. Tablets and capsules are the most useful oral unit dosage because of the readiness of administration. For preparing tablets and capsules, solid pharmaceutical carriers are used.

[0027] Injectable preparations can be prepared using carriers such as distilled water, a salt solution, a glucose solution and a mixture of a salt solution and a glucose solution. The preparation can be prepared in the form of solution, suspension or dispersion according to a conventional method by using a suitable auxiliary.

[0028] Compounds formulae (1) and (2) or a pharmaceutically acceptable salt thereof can be administered orally in the pharmaceutical form described above or parenterally as the injection. The effective dose and administration schedule vary depending on the mode of administration, age, weight, and symptoms of a patient, etc. However, generally, compounds of formulae (1) or (2) or a pharmaceutically acceptable salt thereof are administered in a dose of 1 to 900 mg/60 kg/day, preferably in a dose of 1 to 200 mg/60 kg/day.

[0029] Certain embodiments of the present invention are described in the following examples.

EXAMPLE

Example 1: Tablets

[0030] Tablets having the following composition were prepared in a conventional manner.

[0031] Compound 1 (40 g) was mixed with 286.8 g of lactose and 60 g of potato starch, followed by addition of 120 g of a 10% aqueous solution of hydroxypropyl cellulose. The resultant mixture was kneaded, granulated, and then dried by a conventional method. The granules were refined to give granules used to make tablets. After mixing the granules with 1.2 g of magnesium stearate, the mixture was formed into tablets each containing 20 mg of the active ingredient by using a tablet maker (Model RT-15, Kikusui) having pestles of 8 mm diameter.

[0032] The prescription is shown in Table 2.

Table 2.

Compound 1	20 mg
Lactose	143.4 mg
Potato Starch	30 mg
Hydroxypropyl Cellulose	6 mg
Magnesium Stearate	0.6 mg
	200 mg

Example 2: Capsules

[0033] Capsules having the following composition were prepared in a conventional manner.

[0034] Compound 1 (200 g) was mixed with 995 g of Avicel and 5 g of magnesium stearate. The mixture was put in hard capsules No. 4 each having a capacity of 120 mg by using a capsule filler (Model LZ-64, Zanashi) to give capsules each containing 20 mg of the active ingredient.

[0035] The prescription is shown in Table 3.

Table 3

Compound 1	20 mg
Avicel	99.5 mg
Magnesium Stearate	0.5 mg
	120 mg

Example 3: Injections

[0036] Injections having the following composition were prepared in a conventional manner.

[0037] Compound 1 (1 g) was dissolved in 100 g of purified soybean oil, followed by addition of 12 g of purified egg yolk lecithin and 25 g of glycerin for injection. The resultant mixture was made up to 1,000 ml with distilled water for injection, thoroughly mixed, and emulsified by a conventional method. The resultant dispersion was subjected to aseptic filtration by using 0.2 μm disposable membrane filters, and then aseptically put into glass vials in 2 ml portions to give injections containing 2 mg of the active ingredient per vial.

[0038] The prescription is shown in Table 4.

Table 4

Compound 1	2 mg
Purified Soybean Oil	200 mg
Purified Egg Yolk Lecithin	24 mg
Glycerine for Injection	50 mg
Distilled Water for Injection	1.72 ml
	2.00 ml

Example 4: Anal suppository

[0039] Formulations for rectal administration having the following composition were prepared in a conventional manner.

[0040] Witepsol® H15 (678.8 g, manufactured by Dynamit Nobel, Ltd.) and Witepsol® E75 (290.9 g, manufactured by Dynamit Nobel, Ltd.) were melted at 40 to 50 °C. In the resulting molten mixture were uniformly mixed and dispersed Compound 1 (2.5 g), potassium dihydrogen phosphate (13.6 g) and disodium hydrogen phosphate (14.2 g). The resulting dispersion was poured into plastic suppository molds, and gradually cooled to give anal suppositories containing 2.5 mg of the active ingredient per formulation.

[0041] The prescription is shown in Table 5.

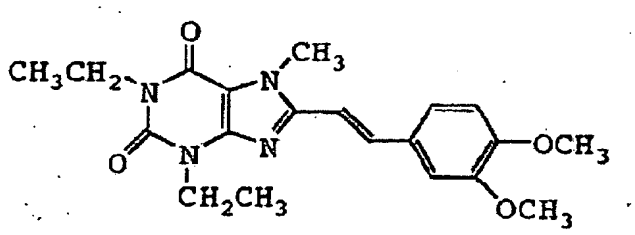
Table 5

Compound 1	2.5 mg
Witepzol H15	678.8 mg
Witepzol E75	290.9 mg
Potassium dihydrogen phosphate	13.6 mg
Disodium hydrogen phosphate	14.2 mg
	1000 mg

[0042] The present invention provides a therapeutic agent for the neurodegenerative disorder brain is chemia.

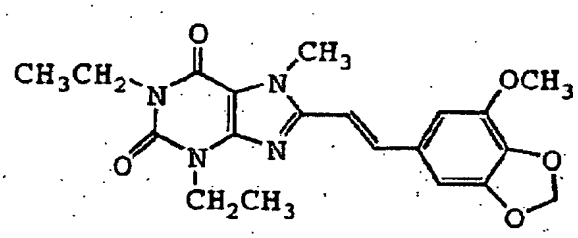
Claims

1. Use of a xanthine derivative selected from a compound of formula (1):



(1)

and a compound of formula (2)



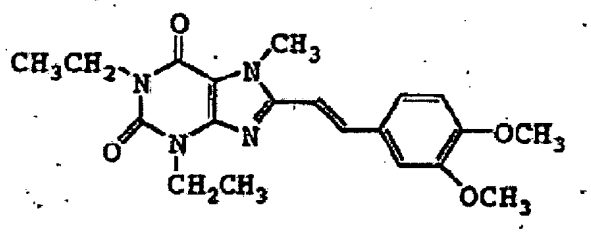
(2)

or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of brain ischemia.

2. Use according to claim 1, wherein the xanthine derivative is represented by formula (1), or a pharmaceutically acceptable salt thereof.

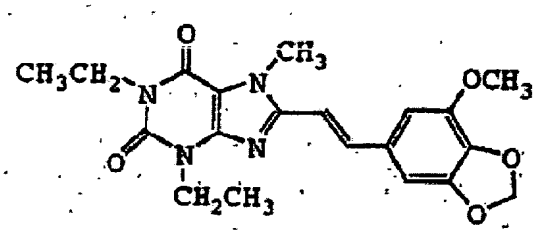
Patentansprüche

1. Verwendung eines Xanthinderivates, ausgewählt aus einer Verbindung der Formel (1):



(1)

und einer Verbindung der Formel (2)



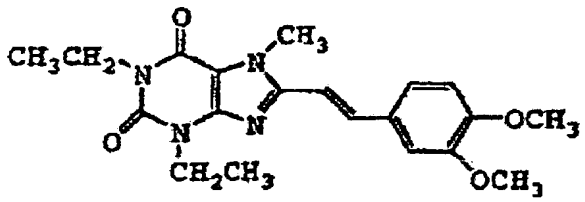
(2)

oder eines pharmazeutisch verträglichen Salzes davon, zur Herstellung eines Medikaments zur Behandlung von Hirnischämie.

2. Verwendung gemäß Anspruch 1, wobei das Xanthinderivat durch die Formel 1 dargestellt ist, oder eines pharmazeutisch verträglichen Salzes davon.

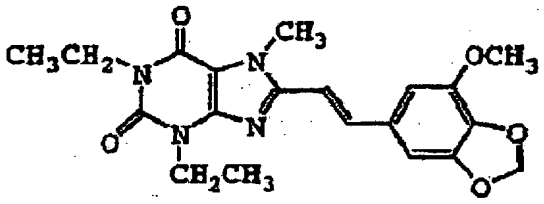
Revendications

1. Utilisation d'un dérivé de xanthine choisi parmi un composé de formule (1) :



(1)

et un composé de formule (2)



(2)

ou un sel pharmaceutiquement acceptable de ceux-ci pour la fabrication d'un médicament destiné au traitement de l'ischémie du cerveau.

2. Utilisation selon la revendication 1, dans laquelle le dérivé de xanthine est représenté par la formule (1), ou un sel pharmaceutiquement acceptable de celui-ci.